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ELECTRON TRANSFER FROM PHOTOREDUCED PRODUCTS OF NAD^+ AND ITS ANALOGUES TO HEMIN IN AQUEOUS SOLUTIONS[1)]

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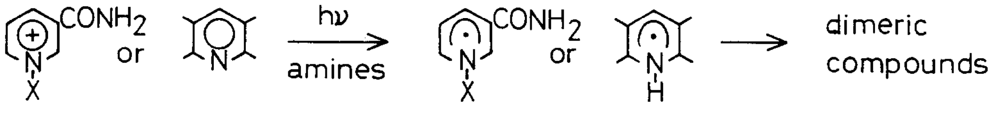
1-Benzyl-3-carbamoylpyridinium chloride (BNA^+) and 3,5-dicarbethoxy-2,6-dimethylpyridine (HE_{ox}) in the aqueous diethylamine solutions are photoreduced, and the 6,6'-dimer of the one-electron reduced BNA^+ is obtained from the former while the latter affords 4,4'-dimeric Hantzsch ester together with 1,2-dihydropyridine.[2)] One-electron transfer from amine to these excited pyridine derivatives also took place when diethylamine was replaced either with ethylenediaminetetraacetic acid (EDTA) or with tetramethylethylenediamine (TMED). In this case, however, 4,4'-dimeric Hantzsch ester was accompanied with 2,2'-dimeric Hantzsch ester (mp 178-179°C (dec.), λ_{max} 282 and 383 nm; yield 34 %) when HE_{ox} was irradiated under stirring in 40%(v/v) $MeCN-H_2O$ containing EDTA and K_2CO_3. 2,2'-Dimeric Hantzsch ester is converted to 4,4'-dimer upon irradiation in MeOH. These results indicate that pyridinyl radicals are formed as common metastable intermediates in the photolysis of BNA^+ and HE_{ox}:



In the present experiments, Hemin was successfully reduced by the use of the metastable pyridinyl radicals generated in the photolysis of BNA^+, HE_{ox}, β-nicotinamideadenine dinucleotide (NAD^+), nicotinamide (NA), and benzyl viologen (BV^{2+}). Either EDTA or TMED was used as the reductant which did not interact with the above pyridine derivatives in the ground states.

Table 1. Photosensitized reduction of Hemin[a)]

Sensitizer	Amine	pH[b)]	Irrad. time, min	Yield of reduced Hemin, %
NAD^+ ($1.0x10^{-3}$ M)	TMED	-	10.0	76
BNA^+ ($1.0x10^{-3}$ M)	EDTA	8.2	5.5	91
BNA^+ ($1.0x10^{-3}$ M)	EDTA	-	2.5	98
BNA^+ ($1.0x10^{-3}$ M)	TMED	-	5.0	98
NA ($2.0x10^{-4}$ M)	TMED	-	8.0	75
HE_{ox} ($5.0x10^{-4}$ M)[c)]	TMED	-	4.0	65
BV^{2+} ($1.0x10^{-4}$ M)	EDTA	7.9	4.0	88
$PFl-BNA^+$ ($8.0x10^{-5}$-$5.0x10^{-4}$ M)[d)]	TMED	-	18.0	85

a) Each solution in a quartz cell (1 cm width) was flushed with Ar gas for 15 min and irradiated with beam of light obtained from a Ushio 500 W super high-pressure Hg lamp. The initial concentrations of Hemin, EDTA, and TMED were 5.0×10^{-5}, 1.25×10^{-2}, and 1.0×10^{-2} M, respectively. In all cases, the solution contained 0.1%(v/v) pyridine and 0.75%(v/v) Triton X-100. Triton X-100 was used to solubilize the H-P complex into aqueous media.

b) The phosphate buffer (1/30 M) was used to adjust the pH. When the pH-value is not indicated, the experiments were carried out by the use of pure water (pH ∿ 6.0).

c) Only in this case, the solution contained 5%(v/v) MeCN.

d) The photolysis was undertaken by irradiating the region (>420 nm), where PFl alone was excited. A Toshiba V-Y42 filter was used to cut off the light at shorter wavelength.

Under the experimental conditions, Hemin was present as Hemin-pyridine complex (H-P complex) and it was not photoreduced in the absence of the pyridine derivatives. By the addition of NAD^+ analogues, however, the H-P complex was efficiently photoreduced as summarized in Table 1. The formation of the reduced Hemin was detected by the increase of characteristic absorption maxima at 526 and 556 nm and was also confirmed by the quantitative reoxidation of Hemin on mixing with air. BNA^+ was the most effective photosensitizer. The concentrations of reduced Hemin in the BNA^+-sensitized photolysis is plotted against the irradiation time in Fig. 1, where the amounts of the formation of the 6,6'-dimer from BNA^+ in the absence of Hemin is also indicated. The rate of the formation of reduced Hemin was significantly higher than that of 6,6'-dimer of BNA^+. In addition, the authentic 6,6'-dimer was found to reduce the H-P complex only with a considerably slow rate in the dark. Analogously, the H-P complex was verified not to be reduced by the stable photoreduced products of NA which were separately prepared in either alkaline ($[OH^-] = 0.2 \sim 10^{-6}$ M) or pure water (pH ∿ 6.0). The sensitized photoreduction of the H-P complex easily took place, however, if both NA and the complex were present in the irradiated solution. All of these data clearly suggest that the main reducing agents in the Hemin reduction are not the stable photoreduced products of the pyridine derivatives. As mentioned in the beginning, the photoreduction of pyridine and pyridinium compounds proceeds *via* the one-electron transfer mechanism, where the pyridinyl radicals are formed as metastable intermediates. Brühlmann and Hayon have studied the radiolysis of NA and NAD^+ analogues in aqueous media and found that the corresponding pyridinyl radicals generated by single electron addition have highly negative kinetic redox potentials ($E^{01} \sim -0.9$ V) and transfer an electron to O_2, methaquinone, riboflavin, or *p*-benzoquinone with diffusion controlled rates ($k \sim 10^9$ $M^{-1}sec^{-1}$).3) The same type of rapid electron-transfer reactions of radiochemically generated NAD have also been reported by Willson.4) Therefore, it is likely that photochemically generated pyridinyl radicals of NAD^+, BNA^+, HE_{ox}, and NA transfer an electron to the H-P complex ($E_{m7.5} \sim 0.1$ V)5) before dimerization. In the case of BV^{2+}, the stable pyridinyl radical (λ_{max} 560 and 600 nm)6) was generated upon irradiation in an Ar-flushed aqueous EDTA solution and reduced the H-P complex as soon as it was mixed with the H-P complex solution in the

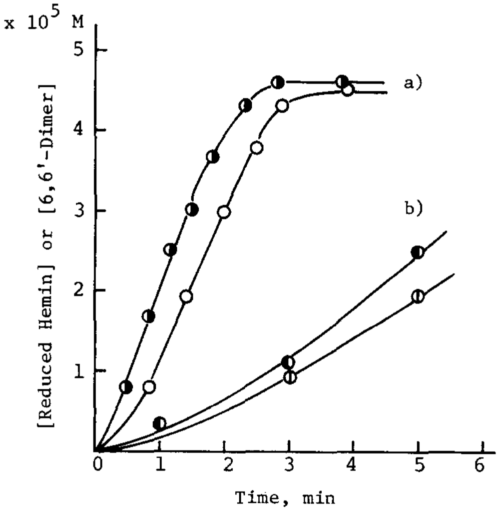


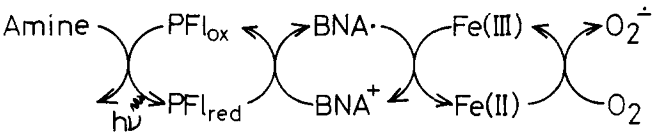
Fig. 1. Formation of reduced Hemin and 6,6'-dimer of BNA^+ along the irradiation in the presence of TMED.

a) Reduction of Hemin in water (-○-) and pH 8.2 phosphate buffer (1/30 M; -◐-). Other reaction conditions were the same as those in Table 1.

b) Formation of 6,6'-dimer under the irradiation of BNA^+ (1.0×10^{-3} M) in Ar-flushed water (-⦶-) and pH 8.2 phosphate buffer (1/30 M; -◐-) containing 0.01 M TMED.

dark. This may be taken as a direct proof for the capability of pyridinyl radical as reducing agent of Hemin.

Unfortunately, NAD^+ and its analogues do not have strong absorption in the visible region. An attempt was made here to utilize visible light by taking into consideration of the fact that the reduction of methyl viologen (E^0 = -0.44 V (pH independent)) to its radical anion is photosensitized by proflavin hemisulfate (PFl) (E^0 = -0.92 V (pH 9-11); λ_{max} 440 nm).[7] The photoreduction of BNA^+ (λ_{max} 263 nm) was analogously sensitized by PFl when a mixture of BNA^+ and PFl in an aqueous TMED solution was irradiated by >420 nm lights. This PFl-sensitized photoreduction of BNA^+ was successfully linked to the reduction of the H-P complex (see Table 1). In the absence of BNA^+, PFl did not reduce the H-P complex at all upon irradiation. Thus, the following redox cycle could be activated by the visible light in the present experiment:



This redox cycle is strongly related to the electron transport in photobiological system and may be adopted as a useful means of transforming energy of light into chemical potentials.

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